Remarks

I. Support for Amendments

The foregoing amendments to the specification are sought solely to delete subject matter from the title of the application as filed, and to insert the formal drawings for this application, which correspond to the informal drawings submitted with the application as filed, into the proper location in the specification. These amendments to the specification add no new matter, and their entry and consideration are respectfully requested.

Support for the foregoing amendments to the claims, and for new claims 158-225, can be found throughout the specification, particularly at pages 6-8; at pages 12-18; at page 30; and throughout the Examples and the drawings. Accordingly, the foregoing amendments to the claims add no new matter, and their entry and consideration are respectfully requested.

II. Status of the Claims

By the foregoing amendments, claims 35-37, 40-42, 57, 58, 69, 115, 133, 141, 149, 151 and 155 have been amended, and new claims 158-225 are sought to be entered. These amendments do not add new matter. Upon entry of the foregoing amendments, claims 35-225 are pending in the application, with claims 35, 78, 115, 151, 159, 187 and 213 being the independent claims.

III. Summary of the Office Action

In the Office Action dated July 16, 2002, the Examiner has made one objection to the specification, and five rejections of the claims. Applicants respectfully offer the following remarks to overcome or traverse each of these elements of the Office Action.

IV. The Objection to the Drawings is Accommodated

In the Office Action at page 2, section 1, the Examiner has objected to the specification for informalities in the drawings, and has required the submission of new formal drawings. By the foregoing amendments, the formal drawings for the present application have been entered into the specification in place of the informal drawings filed with the application on January 30, 2002. Accordingly, this objection has been accommodated; reconsideration and withdrawal are respectfully requested.

V. The Rejection Under 35 U.S.C. § 102(b) Over Knapp

In the Office Action at pages 2-3, section 3, the Examiner has rejected claims 35, 36, 40, 41, 57-59, 60-68, 70, 71 and 74-77 under 35 U.S.C. § 102(b) as being anticipated by U.S. Patent No. 5,159,062 to Knapp *et al.* (Doc. C1 cited on the Form PTO-892 attached to Paper No. 7; hereinafter "Knapp"). Applicants respectfully traverse this rejection.

In making this rejection, the Examiner contends that Knapp discloses every element of the claimed invention, and points to the summary and column 3 of Knapp for specific support for this contention. *See* Paper No. 7 at page 2, section 3. Applicants respectfully disagree. Claim 35 (and thus the remaining claims depending therefrom) recites a method of

producing a nucleic acid molecule comprising: (a) providing a first nucleic acid molecule comprising at least a first gene or portion thereof and at least a first recombination site; (b) providing a second nucleic acid molecule comprising at least a second gene or portion thereof and at least a second recombination site; and (c) forming a mixture *in vitro* between said first and second nucleic acid molecules and at least one recombination protein, under conditions sufficient to cause recombination *in vitro* between said first and second recombination sites, thereby producing a third nucleic acid molecule in which said first and second genes or portions thereof are operably linked to form a functional gene. In contrast, Knapp does not disclose the use of at least two different nucleic acid molecules, each of which comprises at least one recombination site. Instead, the disclosure of Knapp is limited to the use of *one* nucleic acid molecule containing a recombination site (TnPhoA) and contacting this nucleic acid molecule with genomic DNA from *Bordetella pertussis* which does *not* contain a recombination site, to generate a standard cloning vector containing the *Bordetella pertussis* signal sequence (*see* Knapp at cols. 3-4 and in Figure 1). Knapp then goes on to use this cloning vector to construct a further cloning vector (pTrc99C-phoA) and secretion vectors (pSEC-Bp-1, pSEC-Bp-2 and pSEC-Bp-3) using standard restriction/ligation cloning (*see* Knapp at cols. 4-8. Moreover, to the extent that Knapp discloses recombination at all, this reference does not disclose forming a mixture between a first and a second nucleic acid molecule and at least one recombination protein, under conditions sufficient to cause recombination between the first and second recombination sites. Accordingly, Knapp does not disclose at least one element of the claimed invention.

Under 35 U.S.C. § 102, a claim can only be anticipated if every element in the claim is expressly or inherently disclosed in a single prior art reference. *See Kalman v. Kimberly Clark Corp.*, 713 F.2d 760, 771 (Fed. Cir. 1983), *cert. denied*, 465 U.S. 1026 (1984). Since Knapp does not expressly or inherently disclose one or more elements of the claimed invention, this reference cannot and does not anticipate claims 35, 36, 40, 41, 57-59, 60-68, 70, 71 and 74-77. Therefore, reconsideration and withdrawal of the rejection under 35 U.S.C. § 102(b) over Knapp are respectfully requested.

VI. The Rejection Under 35 U.S.C. § 102(b) Over Johnson

In the Office Action at pages 3-4, section 4, the Examiner has rejected claims 35, 36, 40-71, 74-77 and 151-157 under 35 U.S.C. § 102(b) as being anticipated by Published PCT Application No. WO 93/19172 to Johnson *et al.* (Doc. No. AM2, of record; hereinafter "Johnson"). Applicants respectfully traverse this rejection.

A. The Rejection of Claims 151-157

Claim 151 (and thus claims 152-157 that depend ultimately therefrom) recites a method of producing a nucleic acid molecule comprising: (a) providing a first nucleic acid molecule comprising at least one promoter and at least a first *lox*P site; (b) providing a second nucleic acid molecule comprising at least one antibiotic resistance gene or portion thereof and at least a second *lox*P site; and (c) forming a mixture between the first and second nucleic acid molecules and at least one Cre recombination protein, under conditions sufficient to cause recombination between the first and second *lox*P sites, thereby producing a third nucleic acid

molecule in which the promoter and the antibiotic resistance gene or portion thereof are operably linked. In contrast, the disclosure of Johnson relates to the methods for the "shuffling" of heavy (V_H) chains of an antibody molecule by recombination of two nucleic acid molecules, one of which encodes a first V_H antibody chain and the other of which encodes a second, different V_H antibody chain. Johnson provides no disclosure of the operable linkage of a promoter on a first nucleic acid molecule with an antibiotic resistance gene or portion thereof on a second nucleic acid molecule, as recited by present claim 151. Hence, Johnson does not disclose the presently claimed invention.

As the Federal Circuit has held, a claim can only be anticipated by a publication if the publication describes the claimed invention with sufficient enabling detail to place the public in possession of the invention. *See In re Donohue*, 766 F.2d 531, 533 (Fed. Cir. 1985); *see also PPG Industries, Inc. v. Guardian Industries Corp.*, 75 F.3d 1558, 1566 (Fed. Cir. 1996) ("To anticipate a claim, a reference must disclose every element of the challenged claim and enable one skilled in the art to make the anticipating subject matter."). Applicants respectfully submit that Johnson does not disclose the claimed invention. Hence, in view of *Kalman*, *Donohue* and *PPG Industries*, Johnson does not anticipate claims 151-157.

B. The Rejection of Claims 35, 36, 40-71 and 74-77

As noted above, claim 35 (and hence the remaining claims dependent ultimately therefrom that are also rejected over Johnson) recites a method of producing a nucleic acid molecule comprising: (a) providing a first nucleic acid molecule comprising at least a first gene or portion thereof and at least a first recombination site; (b) providing a second nucleic

acid molecule comprising at least a second gene or portion thereof and at least a second recombination site; and (c) forming a mixture *in vitro* between said first and second nucleic acid molecules and at least one recombination protein, under conditions sufficient to cause recombination *in vitro* between said first and second recombination sites, thereby producing a third nucleic acid molecule in which said first and second genes or portions thereof are operably linked to form a functional gene. For at least the reasons noted above, Applicants respectfully submit that Johnson fails to disclose each and every element of the invention of claims 35-77. Moreover, the disclosure of Johnson focuses primarily on *in vivo* recombination methods, wherein the recombination reaction takes place inside of host cells. *See, e.g.*, Johnson at pages 31-32, at pages 37-38, and throughout the Examples, particularly at pages 47-48, 51 and 55-56. Applicants note that Johnson only mentions in passing *in vitro* Cre-*lox*P recombination *in vitro* (*see, e.g.*, Johnson, abstract, at page 27, line 22, at page 33, lines 21-25, and in claim 4 at page 61). However, Johnson provides no experimental details to support such a statement, and all of the protocols in the Examples in Johnson are limited to *in vivo* recombination. Applicants therefore respectfully assert that Johnson does not disclose the claimed invention, and certainly does not enable one of ordinary skill to practice the claimed *in vitro* methods. Hence, in view of *Kalman*, *Donohue* and *PPG Industries*, Johnson does not anticipate claims 35, 36, 40-71 and 74-77, nor new claims 158-225 drawn to *in vitro* methods.

C. Summary

In view of the foregoing remarks, Applicants respectfully assert that claims 35, 36, 40-71, 74-77 and 151-157 are not anticipated by Johnson. Reconsideration and withdrawal of the rejection under 35 U.S.C. § 102(b) over Johnson therefore are respectfully requested.

VII. The Rejection Under 35 U.S.C. § 102(e) Over Demirjian

In the Office Action at pages 4-5, section 5, the Examiner has rejected claims 35[1]-43, 47-50, 54-59, 68-70, 74-83, 86-89, 93-98, 102-119, 122-125, 129-134 and 138-150 under 35 U.S.C. § 102(e) as being anticipated by U.S. Patent No. 5,981,177 to Demirjian *et al.* (Doc. B1 cited on the Form PTO-892 attached to Paper No. 7; hereinafter "Demirjian"). Applicants respectfully traverse this rejection.

A. Claims 78-83, 86-89, 93-98, 102-119, 122-125, 129-134 and 138-150

Claim 78 (and thus claims 79-83, 86-89, 93-98 and 102-114 that depend ultimately therefrom) recites a method of producing a nucleic acid molecule comprising: (a) providing a first nucleic acid molecule comprising a first portion of an antibiotic resistance gene and at least a first recombination site; (b) providing a second nucleic acid molecule comprising a second portion of the antibiotic resistance gene and at least a second recombination site; and

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[1]Applicants note that in the Office Action at page 4, section 5, the Examiner has indicated that "claims 25-43 . . . are rejected . . . ." However, claims 25-34 are not pending in the present application, having been cancelled in Applicants' Preliminary Amendment filed on January 30, 2002. Applicants therefore assume that the designation of claim "25" listed in this section of the Office Action is a typographical error, and that the Examiner actually meant to indicate that claims 35-43 are rejected. Applicants' remarks herein concerning this rejection are based on this assumption.

(c) forming a mixture between the first and second nucleic acid molecules and at least one recombination protein, under conditions sufficient to cause recombination between the first and second recombination sites, thereby producing a third nucleic acid molecule in which the first and second portions of the gene are operably linked to form a functional antibiotic resistance gene. Analogously, claim 115 (and thus claims 116-119, 122-125, 129-134 and 138-150 that depend ultimately therefrom) recites a method of producing a nucleic acid molecule comprising: (a) providing a first nucleic acid molecule comprising at least one promoter and at least a first recombination site; (b) providing a second nucleic acid molecule comprising at least one antibiotic resistance gene or a portion thereof and at least a second recombination site; and (c) forming a mixture between the first and second nucleic acid molecules and at least one recombination protein, under conditions sufficient to cause recombination between the first and second recombination sites, thereby producing a third nucleic acid molecule in which the promoter and the antibiotic resistance gene or portion thereof are operably linked. In contrast, Demirjian does not disclose each and every element of the claimed invention. Therefore, in view of *Kalman*, *Donohue* and *PPG Industries*, Demirjian cannot anticipate 78-83, 86-89, 93-98, 102-119, 122-125, 129-134 and 138-150.

B. Claims 35-43, 47-50, 54-59, 68-70 and 74-77

As noted above, claim 35 (and hence the remaining claims dependent ultimately therefrom that are also rejected over Demirjian) recites a method of producing a nucleic acid molecule comprising: (a) providing a first nucleic acid molecule comprising at least a first gene or portion thereof and at least a first recombination site; (b) providing a second nucleic

acid molecule comprising at least a second gene or portion thereof and at least a second recombination site; and (c) forming a mixture *in vitro* between said first and second nucleic acid molecules and at least one recombination protein, under conditions sufficient to cause recombination *in vitro* between said first and second recombination sites, thereby producing a third nucleic acid molecule in which said first and second genes or portions thereof are operably linked to form a functional gene. In contrast, as discussed above, Demirjian does not disclose each and every element of the claimed invention. Moreover, the reactions disclosed and specifically exemplified in Demirjian take place *in vivo, i.e.*, within the host cells providing the genomic DNA. *See, e.g.*, Demirjian at cols. 13-25, and particularly at cols. 24-25. Applicants note that Demirjian only mentions in passing that recombination may be accomplished *in vitro* (*see, e.g.*, Demirjian, abstract; at col. 6, lines 63-66; at col. 8, lines 7-10; and at col. 10, lines 51-54). However, Demirjian provides no experimental details to support such a statement, and all of the protocols in the Examples in Demirjian are limited to *in vivo* recombination. Applicants therefore respectfully assert that Demirjian does not disclose the claimed invention, and certainly does not enable one of ordinary skill to practice *in vitro* methods of recombination. In view of *Kalman*, *Donohue* and *PPG Industries*, Demirjian therefore cannot anticipate claims 35-43, 47-50, 54-59, 68-70 and 74-77, nor new claims 158-225 which are drawn to *in vitro* methods.

C. Summary

In view of the foregoing remarks, Applicants respectfully assert that claims 35-43, 47-50, 54-59, 68-70, 74-83, 86-89, 93-98, 102-119, 122-125, 129-134 and 138-150 are not

anticipated by Demirjian. Reconsideration and withdrawal of the rejection under 35 U.S.C. § 102(b) over Demirjian therefore are respectfully requested.

VIII. The Rejection Under 35 U.S.C. § 103(a) Over Demirjian and Johnson

In the Office Action at pages 5-7, section 7, the Examiner has rejected claims 35-71, 74-106, 109-142 and 145-157 under 35 U.S.C. § 103(a) as being obvious over Demirjian in view of Johnson. Applicants respectfully traverse this rejection.

In proceedings before the Patent and Trademark Office, the examiner bears the burden of establishing a *prima facie* case of obviousness based upon the prior art. *See In re Piasecki*, 223 USPQ 785, 787-88 (Fed. Cir. 1984). The Examiner can satisfy this burden only by showing some objective teaching in the prior art or that knowledge generally available to one of ordinary skill in the art would lead that individual to combine the relevant teachings of the references in such a way as to produce the invention as claimed. *See In re Fine*, 5 USPQ2d 1596,1598 (Fed. Cir. 1988). Specifically, there must be a reason, suggestion, or motivation in the cited art that would motivate one of ordinary skill to combine the references, and that would also suggest a reasonable likelihood of success in making or using the invention as claimed as a result of that combination. *See In re Dow Chem. Co.*, 837 F.2d 469, 473 (Fed. Cir. 1988). This burden is not met in the present case.

Applicants have discussed the deficiencies of Demirjian above. These deficiencies are not corrected by the disclosure of Johnson, and one of ordinary skill therefore would have found no suggestion or motivation to have combined the disclosures of these references. Thus, the burden required to sustain a *prima facie* case of obviousness has not been met.

In view of the foregoing remarks, Applicants respectfully assert that claims 35-71, 74-106, 109-142 and 145-157 would not have been obvious over the disclosures of Demirjian and Johnson, alone or in combination. Reconsideration and withdrawal of the rejection under 35 U.S.C. § 103(a) over Demirjian in view of Johnson therefore are respectfully requested.

IX. The Rejection Under 35 U.S.C. § 103(a) Over Demirjian, Johnson and Hodges

In the Office Action at pages 5-7, section 7, the Examiner has rejected claims 35-157 under 35 U.S.C. § 103(a) as being obvious over Demirjian in view of Johnson, and further in view of U.S. Patent No. 5,527,695 to Hodges *et al.* (Doc. No. AI1, of record; hereinafter "Hodges"). Applicants respectfully traverse this rejection.

For the reasons discussed above, Demirjian is seriously deficient as a primary reference upon which to base an alleged *prima facie* case of obviousness. For reasons also discussed above, Johnson provides no disclosure that would cure these deficiencies in Demirjian. Finally, Hodges provides no additional disclosure that would have cured the deficiencies in Demirjian and/or Johnson. Hence, one of ordinary skill would have found no suggestion or motivation to have combined the disclosures of these references. Thus, the burden required to sustain a *prima facie* case of obviousness has not been met.

In view of the foregoing remarks, Applicants respectfully assert that claims 35-157 would not have been obvious over the disclosures of Demirjian, Johnson and Hodges, alone or in combination. Reconsideration and withdrawal of this rejection under 35 U.S.C. § 103(a) therefore are respectfully requested.

X. Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider and withdraw all of the outstanding rejections and objections.

It is believed that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt entry and favorable consideration of the foregoing amendments and remarks, and allowance of all pending claims, are earnestly solicited.

Respectfully submitted,

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Version with markings to show changes made

In the Specification:

In the specification, the title of the application and the drawings are amended as follows:

(a) *In the Title:*

The title of the application is amended as follows:

Recombinational Cloning Using [Engineered] Recombination Sites

(b) *In the Drawings:*

The informal drawings submitted with the application as filed are deleted, and the corresponding formal drawings appended hereto are inserted therefor.

In the Claims:

(a) Claims 35-37, 40-42, 57, 58, 69, 115, 133, 141, 149, 151 and 155 are amended as follows:

35. (Once amended) A method of producing a nucleic acid molecule comprising:

(a) providing a first nucleic acid molecule comprising <u>at least</u> a first [portion of a] gene <u>or portion thereof</u> and at least a first recombination site;

(b) providing a second nucleic acid molecule comprising <u>at least</u> a second [portion of said] gene <u>or portion thereof</u> and at least a second recombination site; and

(c) forming a mixture *in vitro* between said first and second nucleic acid molecules and at least one recombination protein, under conditions sufficient to cause recombination *in vitro* between said first and second recombination sites, thereby producing a third nucleic acid molecule in which said first and second genes or portions thereof [of said gene] are operably linked to form a functional gene.

36. (Once amended) The method of claim 35, wherein said first gene or said second gene encodes a selectable marker.

37. (Once amended) The method of claim 35, wherein said first gene or said second gene is an antibiotic resistance gene.

40. (Once amended) The method of claim 35, wherein said [first or second portion of said gene] first gene or portion thereof or said second gene or portion thereof comprises a promoter.

41. (Once amended) The method of claim 35, wherein said [first and second portions of said gene are fragments of a structural gene] first or second genes or portions thereof are structural genes or portion thereof.

42. (Once amended) The method of claim 35, wherein said first gene or said second gene encodes a heterodimeric gene product.

57. (Once amended) The method of claim 35, wherein said first [portion of said] gene or portion thereof is located adjacent to said first recombination site.

58. (Once amended) The method of claim 35, wherein said second [portion of said] gene or portion thereof is located adjacent to said second recombination site.

69. (Once amended) The method of claim 35, wherein said [first or said second portions of said gene are PCR products] first gene or portion thereof or said second gene or portion thereof is a PCR product.

115. (Once amended) A method of producing a nucleic acid molecule comprising:

(a) providing a first nucleic acid molecule comprising at least one promoter and at least a first recombination site;

(b) providing a second nucleic acid molecule comprising at least one antibiotic resistance gene or portion thereof and at least a second recombination site; and

(c) forming a mixture between said first and second nucleic acid molecules and at least one recombination protein, under conditions sufficient to cause recombination between said first and second recombination sites, thereby producing a third nucleic acid molecule in which said promoter and said antibiotic resistance gene or portion thereof are operably linked.

133. (Once amended) The method of claim 115, wherein said antibiotic resistance gene or portion thereof is located adjacent to said second recombination site.

141. (Once amended) The method of claim 115, wherein said [first or said second portions of said gene are PCR products] gene or portion thereof is a PCR product.

149. (Once amended) The method of claim 115, further comprising introducing said nucleic acid molecule into a host cell and expressing said antibiotic resistance gene or portion thereof.

151. (Once amended) A method of producing a nucleic acid molecule comprising:

(a) providing a first nucleic acid molecule comprising at least one promoter and at least a first *lox*P site;

(b) providing a second nucleic acid molecule comprising at least one antibiotic resistance gene or portion thereof and at least a second *lox*P site; and

(c) forming a mixture between said first and second nucleic acid molecules and at least one Cre recombination protein, under conditions sufficient to cause recombination between said first and second *lox*P sites, thereby producing a third nucleic acid molecule in which said promoter and said antibiotic resistance gene or portion thereof are operably linked.

155. (Once amended) The method of claim 151, further comprising introducing said third nucleic acid molecule into a host cell and expressing said antibiotic resistance gene or portion thereof

(b) New claims 158-225 are sought to be entered.